Exhibit K

Leucocyte Typing IV

White Cell Differentiation Antigens

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CD51 (*cont.*)

25 kDa) is non-covalently associated with the VNR β3 chain, which is identical to GPIIIa (CD61 molecule).
Molecular mass 125 kDa (large subunit VNR α-chain); 25 kDa (small subunit VNR α-chain).
Main cellular reactivity Platelets (weak).
Other reactive cells Immunohistology: endothelial cells, smooth muscle cells, fibroblasts, osteoclasts, melanoma cells.
Functions VNR mediates cell adhesion to arg–gly–arp-containing sequences in vitronectin, von Willebrand factor, fibrinogen, thrombospondin.

Selected references

"Fitzgerald, L. A., Ponce, M., Steiner, B., Rall, S. C., Bennett, J. S., and Phillips, D. R. *Biochemistry* 26, 8158 (1987).
"Suzuki, S., Argraves, W. S., Arai, H., Languino, L. R., Piersbacher, M. D., and Ruoslahti, E. *J. biol. Chem.* 262, 14080 (1987).
Lam, S. C. T., Plow, E. F., D'Souza, S. E., Cheresh, D. A., Frelinger III, A. L., and Ginsberg, M. H. *J. biol. Chem.* 264, 3742 (1989).

CDw52

Antibodies assigned at Fourth International Workshop 097; YTH66.9; YTH361.10; YTH34.5.
Other names Campath-1
Molecular structure O-linked carbohydrate as antigenic determinant.
Molecular mass 21-28 kDa (?).
Main cellular reactivity Leucocytes (neutrophils weak, red cells and platelets negative).
Other reactive cells Flow cytometry: eosinophils.
Functions One antibody (YTH361.10) of this series is mitogenic.
Clinical research applications The Campath-1 antibody has been used for prevention of graft vs. host disease in allogeneic bone marrow transplantation.

Selected references

Hale, G., Bright, S., Chumbley, G., Hoang, T., Metcalf, D., Munro, A., and Waldman, H. *Blood* 62, 873 (1983).
Hale, G., Cobbold, S., Waldmann, H., Easter, G., Matejtschuk, P., and Coombs, R. A. *J. Immunol. Meth.* 103, 59 (1987).
Hale, G., Xia, M., and Waldman, H., this volume [N12.1], (*Leucocyte typing IV* (ed. W. Knapp, B. Dörken, W. R. Gilks, E. P. Rieber, R. E. Schmidt, H. Stein, and A. E. G. Kr. von dem Borne). Oxford University Press (1989).)

CD53

Antibodies assigned at Fourth International Workshop HI29; HI36; MEM-53; HD77.
Molecular structure Single-chain glycoprotein.
Molecular mass 32–40 kDa.
Main cellular reactivity Broad pan-leucocyte including normal and neoplastic plasma cells.
Other reactive cells Immunohistology: bone marrow cells, including osteoblasts and osteoclasts.
Functions Stimulation of the oxidative burst.
Clinical research applications CD53 mAb may be a useful tool for the discrimination of haematopoietic neoplasms from sarcomas and melanomas.

Selected references

Stevanova *et al.*, this volume [N8.1], (*Leucocyte typing IV* (ed. W. Knapp, B. Dörken, W. R. Gilks, E. P. Rieber, R. E. Schmidt, H. Stein, A. E. G. Kr. von dem Borne). Oxford University Press (1990).)
Hadam, M. R., this volume [N13], (*Leucocyte typing IV* (ed. W. Knapp, B. Dörken, W. R. Gilks, E. P. Rieber, R. E. Schmidt, H. Stein, and A. E. G. Kr. von dem Borne). Oxford University Press (1989).)

CD54

Antibodies assigned at Fourth International Workshop RR1/1; LB-2; 7F7; 8P5; WEHI-CAMI; OKT27; F2B1.8; My13.
Other names Intercellular adhesion molecule-1 (ICAM-1).
Molecular structure Integral membrane glycoprotein with seven potential N-linked glycosylation sites.
Molecular mass 90 kDa.
Main cellular reactivity Endothelial cells, many cell types upon activation.
Functions ICAM-1 is a lymphokine inducable molecule, has shown to be a ligand for LFA-1 mediated adhesion, a rhinovirus receptor.

Selected references

Dustin, M. L., Staunton, D. E., and Springer, T. A. *Immunol. Today* 9, 213 (1988).
Simmons, D., Makgoba, M. W., and Seed, B. *Nature* 331, 624 (1988).

CD55

Antibodies assigned at Fourth International Workshop 143-30; BRIC110; BRIC128; F2B-7.2.
Other names Decay accelerating factor (DAF).
Molecular structure PI-linked single-chain glycoprotein, alternate splicing generates soluble form (?).
Molecular mass 70 kDa.
Main cellular reactivity All haematopoietic cells and many non-haematopoietic cells.
Functions DAF is involved in limiting complement activation on autologous tissue.
Clinical research applications Diagnosis of paroxysmal nocturnal haemoglobinuria (PNH).

Selected references

Kinoshita, T., Medof, E., Silber, R., and Nussenzweig, V. *J. exp. Med.* 162, 75 (1985).
"Caras, I. W., Davitz, M. A., Rhee, L., Weddell, G., Martin, D. W., Jr., and Nussenzweig, V. *Nature* 325, 545 (1987).
"Medof, M. E., Walter, E. I., Rutgers, J. L., Knowles, D. M., and Nussenzweig, V. *J. exp. Med.* 165, 838 (1987).
Lublin, D. M., Liszwski, M. K., ost, T. W., Arce, M. A., Le Beau, M. M., Rebentisch, M. B., Lemons, R. S., Seya, T., and Atkinson, J. P. *J. exp. Med.* 168, 181 (1988).

CD56

Antibodies assigned at Fourth International Workshop NKH1A; Leu19; FP2-11.14; L185; N901; T-199.
Other names Isoform of N-CAM, NKH1.
Molecular structure Heavily glycosylated 140 kDa isoform of N-CAM.
Molecular mass 220/135 kDa.
Main cellular reactivity NK-cells.
Other reactive cells Flow cytometry: neuroectodermal cells, some T-cell lines.
Functions Homotypic adhesion.
Clinical research applications Identification of NK-cells.

Selected references

Hercend, T., Griffin, J. D., Bensussan, A., Schmidt, R. E., Edson, M. A., Brennan, A., Murray, C., Daley, J. F., Schlossman, S. F., and Ritz, J. *J. clin. Invest.* 75, 932 (1985).
Lanier, L. L., Le, A. M., Civin, C. I., Loken, M. R., and Phillips, J. H. *J. Immunol.* 136, 4480 (1986).
"Cunningham, B. A., Hemperly, J. J., Murray, B. A., Prediger, E. A., Backenbury, R., and Edelman, C. M. *Science* 236, 799 (1987).
Schmidt, R. E., Michon, J. M., Woronicz, J., Schlossman, S. F., Reinherz, E. L., and Ritz, J. *J. clin. Invest.* 79, 305 (1987).

N16 Cluster report: CD56

JÖRG SCHUBERT, LEWIS L. LANIER, and REINHOLD E. SCHMIDT

A new cluster defined by six monoclonal antibodies (mAb) from five different laboratories which have binding specificity to the N-CAM/NKH1 molecule could be established. The molecule has previously been characterized on peripheral blood lymphocytes (PBL) as NKH1. It is expressed on all cells mediating non-MHC restricted cytotoxicity and is, therefore, a pan-NK cell antigen [1]. NKH1A was the first mAb generated by immuniziation against a human NK-clone [2]. It was found to detect the same antigen as the previously published N901 (NKH1) antibody [3]. This antigen has been extensively studied in its relation to NK-cells. It is detected on a subpopulation of approximately 10–15 per cent of human PBL [2–4]. Depletion of NKH1$^+$ cells by complement lysis completely abolishes non-MHC restricted cytotoxicity of PBMNC [2]. Moreover, it can be shown that the vast majority of NK active cells express the NKH1 molecule. FACS sorting of PBL demonstrated all NK cytotoxicity to be contained within the NKH1$^+$ population [5]. The molecule also appears to represent a late activation antigen since it can be induced on various long-term cultured interleukin-2-dependent T-cell clones [6]. These NKH1$^+$ T-cell clones were described as cytotoxic as well as non-cytotoxic.

Lanier *et al.* [7] presented data indicating that the NKH1 molecule is identical to the 140-kDa isoform of human neuronal cellular adhesion molecule N-CAM. In previous studies it has been demonstrated that the N-CAM molecule exists in at least three different isoforms [8]. A 120-kDa molecule which has a PI-linked membrane anchor, a 140 and a 160-kDa isoform have been described [8–10]. The isoforms are products of different RNA splicing from a single gene located on chromosome 11 [11–13] and have been demonstrated to play a role in neuronal homotypic cell adhesion and cell differentiation during embryogenesis [14].

Immunoprecipitation from NK-cells and from neuroblastoma using either polyvalent rabbit antisera against human N-CAM or the NKH1-specific mAb Leu-19 and subsequent deglycosylation revealed an identical band of 140 kDa [7]. In neuronal tissue three different isoforms can be detected. In contrast, NK-cells express the 140-kDa molecule only. In both cell types the molecule contains about 25–30 per cent of N-linked sugars and sialic acid.

Three mAb have been submitted to the new antibody panel as recognizing the NKH1 antigen: N3 (Leu-19), N9 (N901), and N77 (NKH1A). As control antibody Leu-19 was utilized as a duplicate (N3 = N79) in the panel. This reagent proved to be very useful in evaluating functional studies. During the Workshop pre-screening procedure three additional antibodies from the new antibody panel submitted with unknown specificities were assigned to the N-CAM/NKH1 group: N21 (FP2-11.14), N63 (T-199), and N126 (L185). On evaluating the phenotypic, biochemical, and histological data the six antibodies define a new cluster. The phenotypic pattern of the antibodies is shown in Fig. 1. From the histological data it was suggested that, in addition to N-CAM/NKH1 specificity, another reactivity might be detected in N63 (T-199). Binding specificity of the grouped antibodies was also studied biochemically. Immunoprecipitation demonstrated a single band of approximately 160 kDa. This precipitation was performed by Perussia from cultured NK-cells. Geisberg *et al.* [N21.1] detected two bands of 220 and 135 kDa when using LGL-leukaemia cells as target (Table 1). Antibodies NKH1A and L185, both of IgM isotype, failed to precipitate specific bands. The differences in molecular weight may correspond to differences in glycosylation of the molecules on the different targets used for precipitation. The additional band of 135 kDa from the precipitation of LGL-leukaemia cells obviously represents the unglycosylated core protein.

For epitope mapping, cross-blocking studies were performed. Using the mAb grouped according to their binding specificity to NKH1, three different epitopes on the antigen were identified: NKH1a defined by antibodies, N3 (Leu-19) and N21 (FP2-11.14); NKH1b by N9 (N901) and N77 (NKH1A); and NKH1c by N63 (T-199) and N126 (L185) [Schubert *et al.*, N16.1]. In contrast, using rosetting techniques with chromium-chloride-treated erythrocytes, two groups of epitopes were described by Pietsch and Hadam (N16.2). They grouped antibodies N3 (Leu-19), N21 (FP2-11.14), N63 (T-199), and N126 (L185) as completely blocking rosetting of mAb T-199. N9 (N901) and N77 (NKH1A) only partially blocked T-199. Data from both groups provided evidence that antibodies N9 (N901) and N77 (NKH1A) recognize the same epitope on the NKH1 antigen. The differences observed by the two groups may be due to different methods used. Whereas our group tested four of the NKH1

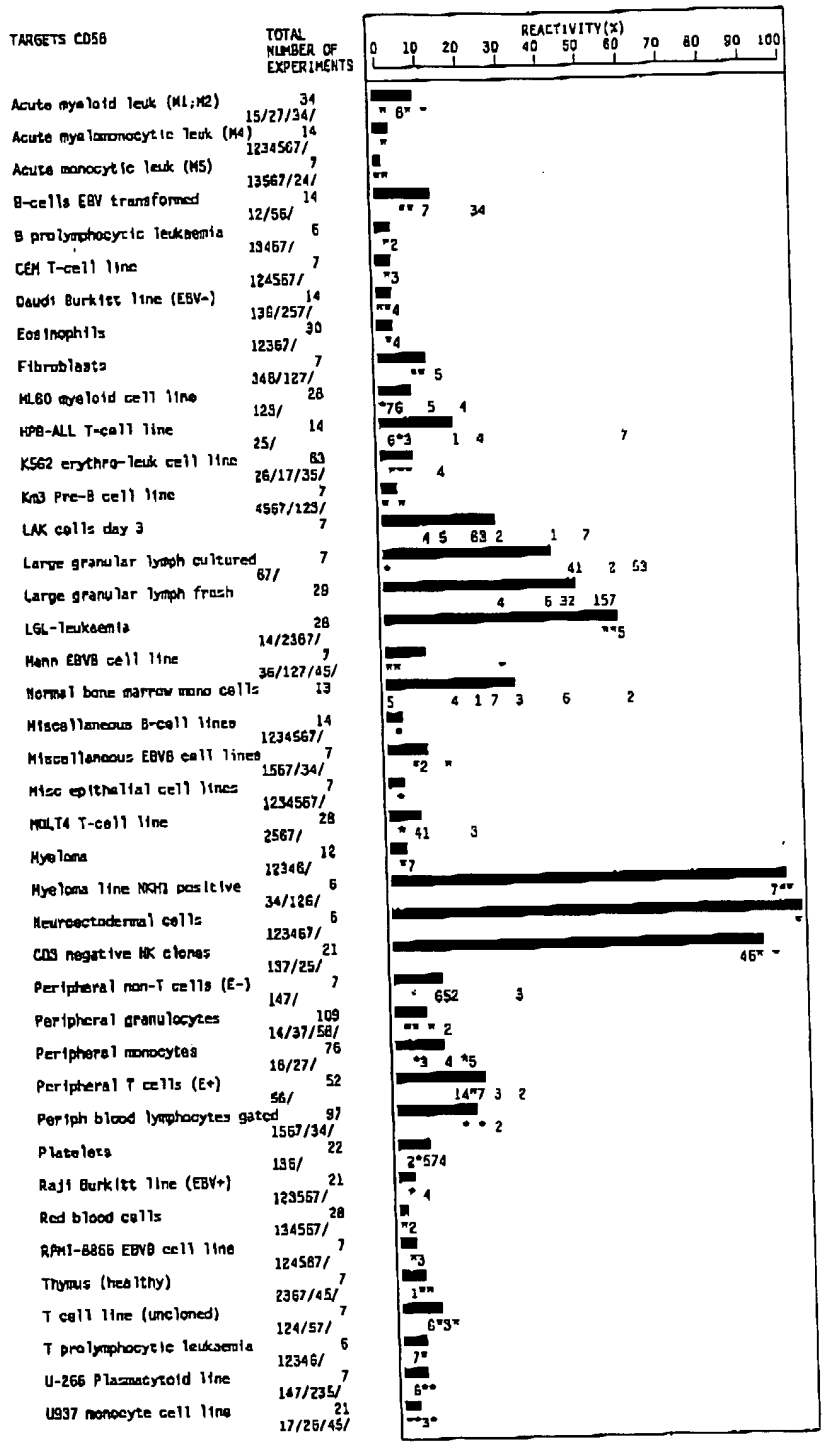

Fig. 1. Mean reactivity profiles of CD56 mAb.

Table 1. CD56 antibodies

Workshop no.	mAb name	Submitter	Species*	Isotype		Com†		W‡	F§	Molecular weight (kDa)¶		Subm. spec.
						R	H			Submitter	Workshop	
N003	Leu-19	Lanier	M	Ig1	k	No	No			155	220/135	Unique
N009	N901	Griffin	M	IgG1		No	No		Yes	200	135	Unique
N021	FP2-11.14	Poncelet	M	IgG2a		Yes	nd		No		220/135	Unique
N063	T-199	Feickert	M	IgG1	k	nd	nd		Yes		220/135	Unique
N077	NKH1A	Ritz	M	IgM		Yes	nd			200		Unique
N126	L185	Lanier	M	IgM	k	Yes	nd			155		Unique

*M, mouse.
†Com, complement binding: R, rabbit; H, human; nd, not done; No, does not fix C; Yes, does fix C.
‡W, Western blotting.
§F, formalin fixation.
¶Molecular weight of multiple polypeptide chains are separated by a solidus (/).

antibodies as second reagents, Pietsch and Hadam used only N63 (T-199) as a second antibody.

Consistent with prior results [6], cultured human thymocytes acquire NKH1 expression and non-MHC restricted cytotoxicity [Heiken *et al.*, N16.4]. Increasing expression of NKH1 antigen was seen with duration of thymocyte culture and the expression of NKH1 correlated with activation of NK- and T-lymphocytes. By analysing stimulated thymocytes on a clonal level, the NKH1 antigen was detected on cytotoxic as well as on non-cytotoxic clones.

Several laboratories reported strong reactivity of N-CAM/NKH1 panel antibodies with tumour cells derived from the neuroectoderm, such as neuroblastoma, medulloblastoma, retinoblastoma, phaeochromocytoma, some melanomas, and small-cell lung carcinomas [Feickert *et al.*, N16.3]. Niedecken described N-CAM/NKH1 antibodies as also reacting with peripheral automonous nerve fibres. A cDNA has been cloned for human N-CAM [10]. Unfortunately, only one mAb, Leu-19, was tested on transfectants, and was found to be positive.

The role of the N-CAM/NKH1 molecule in NK-cell function or activation could not be elucidated during this Workshop. Various laboratories tested the new antibody panel for induction or blocking of cytotoxic activity, or proliferation of NK-cells. None of the antibodies with specificity to NKH1 could significantly affect NK-cell function.

In summary, the CD56 cluster defined by six mAb from five different laboratories could be established during this Workshop. This could be based upon their identical phenotypic reactivity pattern, and molecular weight.

Acknowledgements

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References

1. Ritz, J., Schmidt, R. E., Michon, J., Hercend, T., and Schlossman, S. F. *Adv. Immunol.* **42**, 181 (1988).
2. Hercend, T., Griffin, J. D., Bensussan, A., Schmidt, R. E., Edson, M. A., Brennan, A., Murray, C., Daley, J. F., Schlossman, S. F., and Ritz, J. *J. clin. Invest.* **75**, 932 (1985).
3. Griffin, J. D., Hercend, T., Beveridge, R., and Schlossman, S. F. *J. Immunol.* **130**, 2947 (1983).
4. Lanier, L. L., Le, A. M., Civin, C. I., Loken, M. R., and Phillips, J. H. *J. Immunol.* **136**, 4480 (1986).
5. Schmidt, R. E., Michon, J. M., Worinicz, J., Schlossman, S. F., Reinherz, E. L., and Ritz, J. *J. clin. Invest.* **79**, 305 (1987).
6. Lanier, L. L., Le, A. M., Ding, A., Evans, E. L., Krensy, A. M., Clayberger, C., and Phillips, J. H. *J. Immunol.* **138**, 2019 (1987).
7. Lanier, L. L., Testi, R., Bindl, J., and Phillips, J. M. *J. exp. Med.* **169**, 2233 (1989).
8. Cunningham, B. A., Hemperly, J. J., Murray, B. A., Prediger, E. A., Brackenbury, R., and Edelman, G. M. *Science* **236**, 799 (1987).
9. Hempley, J. J., Edelman, G. M., and Cunningham, B. A. *Proc. nat. Acad. Sci, USA* **83**, 9822 (1986).
10. Barton, C. H., Dickson, G., Gower, H. J., Rowett, L. H., Putt, W., Elsom, V., Moore, S. E., Goridis, C., and Walsh, F. S., *Development* **104**, 165 (1988).
11. Walsh, F. S. *Neurochem. Internat.* (in press).
12. Nguyen, C., Mattei, M. G., Mattei, J. M., Santoni, M. J., Goridis, C., and Jordan, B. R. *J. Cell Biol.* **102**, 711 (1986).
13. Owens, G. C., Edelman, G. M., and Cunningham, B. A. *Proc. nat. Acad. Sci., USA* **84**, 294 (1987).
14. Edelman, G. M. *Science* **219**, 450 (1983).

N16.1 Heterogeneity of the NKH1 molecule

JÖRG SCHUBERT, PETER UCIECHOWSKI, PATRICIA DELANY, KATHRIN WORDELMANN, and REINHOLD E. SCHMIDT

Six mAb from the New Antibody Panel have been identified as recognizing the NKH1 antigen according to their phenotypic pattern: N3 (Leu-19), N9 (N901), N21 (FP2-11.14), N63 (T-199), N77 (NKH1A), and N126 (L185). They all bind to various preparations of NK-cells and to several tumour cell lines of myeloid and neuroectodermal origin [Schubert *et al.*, N16].

We tested the available Workshop antibodies with NKH1 specificity for functional effects on cloned human NK-cell lines. None of the NKH1 antibodies could